

Claims

1. Protein L having the ability to bind to the light chains of immunoglobulins, characterized in that the protein L has the following amino acid sequence:

— B1

Ala	Val	Glu	Asn	Lys	Glu	Glu	Thr	Pro	Glu	Thr	Pro	Glu	Thr	Asp	Ser
1				5					10					15	
Glu	Glu	Glu	Val	Thr	Ile	Lys	Ala	Asn	Leu	Ile	Phe	Ala	Asn	Gly	Ser
			20					25					30		
Thr	Gln	Thr	Ala	Glu	Phe	Lys	Gly	Thr	Phe	Glu	Lys	Ala	Thr	Ser	Glu
		35					40					45			
Ala	Tyr	Ala	Tyr	Ala	Asp	Thr	Leu	Lys	Lys	Asp	Asn	Gly	Glu	Tyr	Thr
	50					55					60				
Val	Asp	Val	Ala	Asp	Lys	Gly	Tyr	Thr	Leu	Asn	Ile	Lys	Phe	Ala	Gly
65					70				75						80
Lys	Glu	Lys	Thr	Pro	Glu	Glu	Pro	Lys	Glu	Glu	Val	Thr	Ile	Lys	Ala
				85					90					95	
Asn	Leu	Ile	Tyr	Ala	Asp	Gly	Lys	Thr	Gln	Thr	Ala	Glu	Phe	Lys	Gly
			100					105					110		
Thr	Phe	Glu	Glu	Ala	Thr	Ala	Glu	Ala	Tyr	Arg	Tyr	Ala	Asp	Ala	Leu
		115					120					125			
Lys	Lys	Asp	Asn	Gly	Glu	Tyr	Thr	Val	Asp	Val	Ala	Asp	Lys	Gly	Tyr
	130					135					140				
Thr	Leu	Asn	Ile	Lys	Phe	Ala	Gly	Lys	Glu	Lys	Thr	Pro	Glu	Glu	Pro
145					150				155					160	
Lys	Glu	Glu	Val	Thr	Ile	Lys	Ala	Asn	Leu	Ile	Tyr	Ala	Asp	Gly	Lys
				165					170					175	
Thr	Gln	Thr	Ala	Glu	Phe	Lys	Gly	Thr	Phe	Glu	Glu	Ala	Thr	Ala	Glu
			180					185					190		
Ala	Tyr	Arg	Tyr	Ala	Asp	Leu	Leu	Ala	Lys	Glu	Asn	Gly	Lys	Tyr	Thr
		195					200					205			
Val	Asp	Val	Ala	Asp	Lys	Gly	Tyr	Thr	Leu	Asn	Ile	Lys	Phe	Ala	Gly
	210					215					220				

— B3

— B4
 Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala
 225 230 235 240
 5 Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly
 245 250 255
 Thr Phe Ala Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Leu Leu
 260 265 270
 10 Ala Lys Glu Asn Gly Lys Tyr Thr Ala Asp Leu Glu Asp Gly Gly Tyr
 275 280 — B5 285
 Thr Ile Asn Ile Arg Phe Ala Gly Lys Lys Val Asp Glu Lys Pro Glu
 290 295 300
 Glu

15

and variants, subfragments, multiples or mixtures of the domains B1-B5 having the same binding properties.

2. DNA-sequence, characterized in that
 20 it codes for the protein according to Claim 1 and has
 the following nucleotide sequence:

GCG GTA GAA AAT AAA GAA GAA ACA CCA GAA ACA CCA GAA ACT GAT TCA 28
 25 GAA GAA GAA GTA ACA ATC AAA GCT AAC CTA ATC TTT GCA AAT GGA AGC 96
 ACA CAA ACT GCA GAA TTC AAA GGA ACA TTT GAA AAA GCA ACA TCA GAA 144
 GCT TAT GCG TAT GCA GAT ACT TTG AAG AAA GAC AAT GGA GAA TAT ACT 192
 30 GTA GAT GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA 240
 AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA 288
 AAC TTA ATC TAT GCA GAT GGA AAA ACA CAA ACA GCA GAA TTC AAA GGA 336
 ACA TTT GAA GAA GCA ACA GCA GAA GCA TAC AGA TAT GCA GAT GCA TTA 384
 35 AAG AAG GAC AAT GGA GAA TAT ACA GTA GAC GTT GCA GAT AAA GGT TAT 432
 ACT TTA AAT ATT AAA TTT GCT GGA AAA GAA AAA ACA CCA GAA GAA CCA 480
 AAA GAA GAA GTT ACT ATT AAA GCA AAC TTA ATC TAT GCA GAT GGA AAA 528

ACA CAA ACA GCA GAA TTC AAA GGA ACA TTT GAA GAA GCA ACA GCA GAA 576
 GCA TAC AGA TAT GCT GAC TTA TTA GCA AAA GAA AAT GGT AAA TAT ACA 624
 5 GTA GAC GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA 672
 AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA 720
 AAC TTA ATC TAT GCA GAT GGA AAA ACT CAA ACA GCA GAG TTC AAA GGA 768
 10 ACA TTT GCA GAA GCA ACA GCA GAA GCA TAC AGA TAC GCT GAC TTA TTA 816
 GCA AAA GAA AAT GGT AAA TAT ACA GCA GAC TTA GAA GAT GGT GGA TAC 864
 ACT ATT AAT ATT AGA TTT GCA GGT AAG AAA GTT GAC GAA AAA CCA GAA 912
 15 GAA TAATAA 921

3. A hybrid protein, characterized in
 that it includes one or more of the B1-B5-domains ac-
 20 cording to Claim 1 which bind to the light chains in
 immunoglobulins of all classes, and domains which bind
 to heavy chains in immunoglobulin G.

4. A hybrid protein according to Claim 3, characterized
 25 in that the domains which bind to
 heavy chains in immunoglobulin G are chosen from among
 the C1- and C2-domains in protein G or from among any
 other functionally similar proteins which bind to heavy
 chains in immunoglobulin G, and variants, subfragments,
 30 multiples or mixtures thereof having the same binding
 properties.

5. A hybrid protein according to Claim 4, characterized
 35 in that the hybrid protein has the
 following amino acid sequence:

	Ala Val Glu Asn Lys Glu Glu Thr Pro Glu Thr Pro Glu Thr Asp Ser	
	5	10 15
5	Glu Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Phe Ala Asn Gly Ser	
	20	25 30
	Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Lys Ala Thr Ser Glu	
	35	40 45
10	Ala Tyr Ala Tyr Ala Asp Thr Leu Lys Lys Asp Asn Gly Glu Tyr Thr	
	50	55 60
	Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly	
	65	70 75 80
	Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala	
15	85	90 95
	Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly	
	100	105 110
	Thr Phe Glu Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Ala Leu	
	115	120 125
20	Lys Lys Asp Asn Gly Glu Tyr Thr Val Asp Val Ala Asp Lys Gly Tyr	
	130	135 140
	Thr Leu Asn Ile Lys Phe Ala Gly Lys Glu Lys Thr Pro Glu Glu Pro	
	145	150 155 160
25	Lys Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Tyr Ala Asp Gly Lys	
	165	170 175
	Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Glu Ala Thr Ala Glu	
	180	185 190
	Ala Tyr Arg Tyr Ala Asp Leu Leu Ala Lys Glu Asn Gly Lys Tyr Thr	
30	195	200 205
	Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly	
	210	215 220
	Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala	
	225	230 235 240
35	Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly	
	245	250 255

Thr Phe Ala Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Leu Leu
 260 265 270
 5 Ala Lys Glu Asn Gly Lys Tyr Thr Ala Asp Leu Glu Asp Gly Gly Tyr
 275 280 285
 Thr Ile Asn Ile Arg Phe Ala Gly Lys Lys Val Asp Glu Lys Pro Glu
 290 295 300
 10 Glu Pro Met Asp Thr Tyr Lys Leu Ile Leu Asn Gly Lys Thr Leu Lys
 305 310 315 320
 Gly Glu Thr Thr Thr Glu Ala Val Asp Ala Ala Thr Ala Glu Lys Val
 325 330 335
 Phe Lys Gln Tyr Ala Asn Asp Asn Gly Val Asp Gly Glu Trp Thr Tyr
 15 340 345 350
 Asp Asp Ala Thr Lys Thr Phe Thr Val Thr Glu Lys Pro Glu Val Ile
 355 360 365
 Asp Ala Ser Glu Leu Thr Pro Ala Val Thr Thr Tyr Lys Leu Val Ile
 370 375 380
 20 Asn Gly Lys Thr Leu Lys Gly Glu Thr Thr Thr Lys Ala Val Asp Ala
 385 390 395 400
 Glu Thr Ala Glu Lys Ala Phe Lys Gln Tyr Ala Asn Asp Asn Gly Val
 405 410 415
 25 Asp Gly Val Trp Thr Tyr Asp Asp Ala Thr Lys Thr Phe Thr Val Thr
 420 425 430
 Glu Met

30 and variants, subfragments, multiples or mixtures of the
 domains B1-B5 having the same binding properties.

6. DNA-sequence, characterized in that
 it codes for a protein according to Claim 5 and has the
 35 following nucleotide sequence:

	GCG GTA GAA AAT AAA GAA GAA ACA CCA GAA ACA CCA GAA ACT GAT TCA	48
	GAA GAA GAA GTA ACA ATC AAA GCT AAC CTA ATC TTT GCA AAT GGA AGC	96
	ACA CAA ACT GCA GAA TTC AAA GGA ACA TTT GAA AAA GCA ACA TCA GAA	144
	GCT TAT GCG TAT GCA GAT ACT TTG AAG AAA GAC AAT GGA GAA TAT ACT	192
5	GTA GAT GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA	240
	AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA	288
	AAC TTA ATC TAT GCA GAT GGA AAA ACA CAA ACA GCA GAA TTC AAA GGA	336
10	ACA TTT GAA GAA GCA ACA GCA GAA GCA TAC AGA TAT GCA GAT GCA TTA	384
	AAG AAG GAC AAT GGA GAA TAT ACA GTA GAC GTT GCA GAT AAA GGT TAT	432
	ACT TTA AAT ATT AAA TTT GCT GGA AAA GAA AAA ACA CCA GAA GAA CCA	480
	AAA GAA GAA GTT ACT ATT AAA GCA AAC TTA ATC TAT GCA GAT GGA AAA	528
15	ACA CAA ACA GCA GAA TTC AAA GGA ACA TTT GAA GAA GCA ACA GCA GAA	576
	GCA TAC AGA TAT GCT GAC TTA TTA GCA AAA GAA AAT GGT AAA TAT ACA	624
	GTA GAC GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA	672
20	AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA	720
	AAC TTA ATC TAT GCA GAT GGA AAA ACT CAA ACA GCA GAG TTC AAA GGA	768
	ACA TTT GCA GAA GCA ACA GCA GAA GCA TAC AGA TAC GCT GAC TTA TTA	816
	GCA AAA GAA AAT GGT AAA TAT ACA GCA GAC TTA GAA GAT GGT GGA TAC	864
25	ACT ATT AAT ATT AGA TTT GCA GGT AAG AAA GTT GAC GAA AAA CCA GAA	912
	GAA CCC ATG GAC ACT TAC AAA TTA ATC CTT AAT GGT AAA ACA TTG AAA	960
	GGC GAA ACA ACT ACT GAA GCT GTT GAT GCT GCT ACT GCA GAA AAA GTC	1008
30	TTC AAA CAA TAC GCT AAC GAC AAC GGT GTT GAC GGT GAA TGG ACT TAC	1056
	GAC GAT GCG ACT AAG ACC TTT ACA GTT ACT GAA AAA CCA GAA GTG ATC	1104
	GAT GCG TCT GAA TTA ACA CCA GCC GTG ACA ACT TAC AAA CTT GTT ATT	1152
	AAT GGT AAA ACA TTG AAA GGC GAA ACA ACT ACT AAA GCA GTA GAC GCA	1200
35	GAA ACT GCA GAA AAA GCC TTC AAA CAA TAC GCT AAC GAC AAC GGT GTT	1248
	GAT GGT GTT TGG ACT TAT GAT GAT GCG ACT AAG ACC TTT ACG GTA ACT	1296
	GAA ATG TAATAA	1308

7. DNA-sequence, characterized in that it codes for a protein according to Claim 3 and 4.

5 8. DNA-sequence, characterized in that it hybridizes to the DNA-sequence of Claim 2, 6 or 7 under conventional conditions and codes for a protein which has the same binding properties as the protein according to any one of Claims 1 and 3-5.

10 9. A plasmid vector, characterized in that it includes a DNA-sequence according to any one of Claims 2 and 6-8, preferably the vector pHDLG or pHDL.

15 10. A host cell, characterized in that it is transformed with the hybrid plasmid according to Claim 9, in particular a host which belongs to the species E. coli, particularly E. coli LE392, or Bacillus subtilis, Saccharomyces cerevisiae, preferably Id. Ref. DSSM E. coli LE392 pHDL and E. coli LE392/pHDLG respectively.
20

11. A method for producing a protein according to Claims 1 and 3-5, characterized by cultivating a host cell according to Claim 10 under suitable
25 conditions; accumulating the protein in the culture or lysing the cells and extracting the protein therefrom.

12. A reagent apparatus for binding, separating and identifying immunoglobulins, characterized
30 in that it includes a protein according to any one of Claims 1 and 3-5.

13. A composition, characterized in that it includes a protein according to any one of Claims 1
35 and 3-5, and optionally additives or carriers.

14. A pharmaceutical composition, c h a r a c -
t e r i z e d in that it includes a protein according
to any one of Claims 1 and 3-5, and optionally a pharma-
ceutically acceptable carrier or extender.